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Paradoxes in longevity: sequence analysis of mtDNA haplogroup J in centenarians

Giuseppina Rose¹, Giuseppe Passarino¹, Giuseppina Carrieri¹, Katia Altomare¹,
Valentina Greco¹, Stefano Bertolini², Massimiliano Bonafè³, Claudio Franceschi^{3,4} and
Giovanna De Benedictis^{*,1}

¹Department of Cell Biology, University of Calabria, Rende, Italy; ²Department of Internal Medicine, University of Genova, Genova, Italy; ³Department of Experimental Pathology, University of Bologna, Bologna, Italy; ⁴National Institute for Aging Research (INRCA), Ancona, Italy

Previous studies have shown that mitochondrial DNA (mtDNA) haplogroup J is significantly over-represented in healthy centenarians with respect to younger controls, thus suggesting that this haplogroup predisposes to successful aging and longevity. On the other hand, the same haplogroup is reported to have elevated frequency in some complex diseases. To verify if centenarians clustered in a particular lineage within J we have sequenced the D-loop region from 18 centenarians and 18 younger controls, previously characterized to be J. Then the entire mtDNA molecule was sequenced in a sub-sample of nine centenarians to find possible functional mutations associated with haplogroup J in successful aging. No clustering of the J haplogroup mtDNA from centenarians was observed. In addition, most of the mutations found are known as disease-associated mutations. The general picture that emerges from the study is that the J haplogroup of centenarians is surprisingly similar to that found in complex diseases, as well as in Leber Hereditary Optic Neuropathy. This finding implies that the same mutations could predispose to disease or longevity, probably according to individual-specific genetic backgrounds and stochastic events. This data reveals another paradox of centenarians and confirms the complexity of the longevity trait. *European Journal of Human Genetics* (2001) 9, 701–707.

Keywords: aging; longevity; mitochondrial DNA

Introduction

Much evidence has accumulated that mitochondrial DNA (mtDNA) is associated with the aging process.¹ It was first recognized that somatic mutations accumulate during aging,² suggesting that dysfunction of mtDNA, which is essential in the energy balance of the cell, but which also is responsible for most of the endogenous free radicals and for apoptosis regulation, may play a role in the pathophysiology of aging and senescence.³ On the other hand, increasing evidences indicate that mtDNA inherited variability plays a

role in successful aging and longevity.^{4–7} Recently it was observed that in Italy the population pool of mtDNA haplogroups is different between healthy centenarians and young individuals, the main difference being sustained by the J haplogroup whose frequency increased from 2% in controls to about 20% in male centenarians.⁸ This observation prompted us to suggest that haplogroup J may be associated with longevity. This inference was subsequently confirmed by a different statistic and demographic approach to the data that takes into account the mortality trajectories of the studied population.⁹ In addition, this finding has been recently confirmed in human longevity studies carried out in Northern Irish population, where a J subgroup was found to be over-represented in centenarians with respect to younger controls.¹⁰ Intriguingly, J haplogroup is reported to boost the effect of mutations causing a mitochondrial disease, the

*Correspondence: G De Benedictis, Department of Cell Biology, University of Calabria, 87030 Rende, Italy.

Tel. +39 0984 492932; Fax +39 0984 492911;

E-mail g.debenedictis@unical.it

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Leber Hereditary Optic Neurophaty (LHON),¹¹ and to contribute to optic neuritis in multiple sclerosis patients.¹² In order to clarify this apparent paradox, first we studied the D-loop region (HVS-I) in mtDNA from 18 healthy centenarians (category A according to the classification given by Franceschi *et al.*¹³) and 18 younger controls previously characterized as J, to verify if centenarians clustered in a particular lineage within J. We then sequenced the whole mtDNA coding region in a sub-sample of nine centenarians, to investigate possible functional mutations associated with haplogroup J in successful aging.

Materials and methods

Subjects

Our samples consisted of 18 healthy centenarians (7 males and 11 females, category A according to the classification given by Franceschi *et al.*¹³) and 18 healthy younger controls (8 males and 10 females) recruited in the previous study.⁸

PCR

Total DNA was extracted from blood buffy coats following standard procedures. PCR amplification was performed on 300 ng of total DNA in 100 μ l of reaction mixture containing 200 μ M of each dNTP, 30 pmol of each primer, 2.5 U of Taq

DNA polymerase and 1.5 mM MgCl₂. The reaction was carried out for a total of 35 cycles in a GeneAmp PCR System 9600 (Perkin Elmer).

The whole mtDNA was amplified in eleven overlapping fragments using the primer pairs and amplification conditions previously described.¹⁴

Sequencing

PCR-amplified fragments were purified using a QIAquick PCR purification Kit (Qiagen).

The sequences were determined by a fluorescence-based automated direct sequencing technique using a model 310 DNA sequencer with BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems).

Sequencing reaction mixtures contained 8 μ l of Terminator Ready Reaction mix, 300 ng of template, 3.2 pmol of primer in a total volume of 20 μ l. Cycle sequencing was carried out for 25 cycles at 96°C for 10 s, 50°C for 5 s, 60°C for 4 min in a GenAmp PCR system 9600. The extension products were purified using the Centri-Sep[®] spin columns (Princeton Separations).

Network analysis

The phylogenetic relationships among the sequences were calculated with the program Network 2.0.^{15,16}

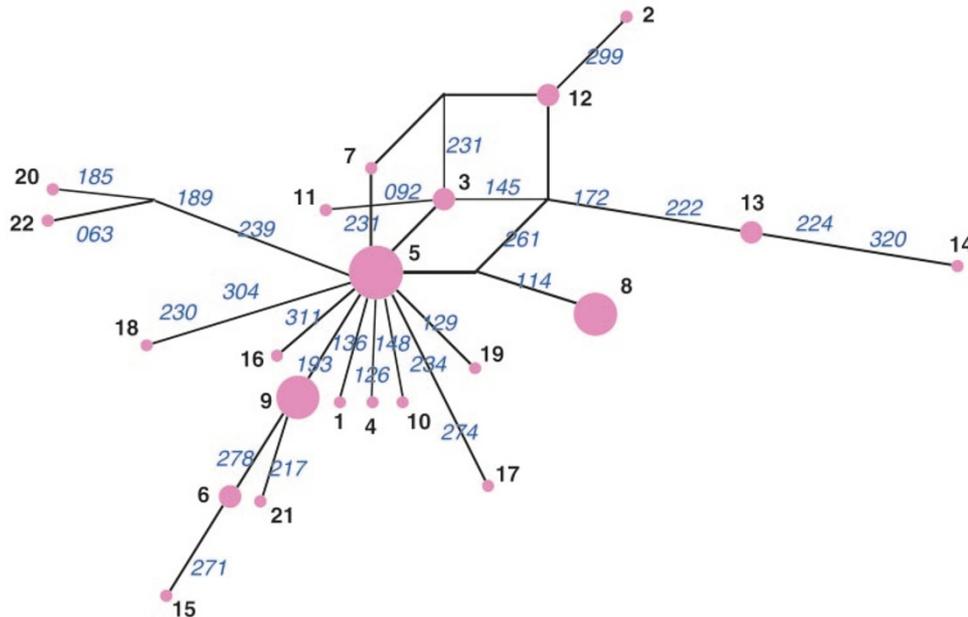


Figure 1 Median Network of the HVSI haplotypes. The mutations are shown in italics. The position of the mutations are given according to Anderson *et al.*¹⁸ less 16 000. The haplotype identification number is shown in bold. The radius of the circles indicating each haplotype is proportional to the number of individuals. Controls and Centenarians (with the respective identification number) are denoted C and L respectively. The samples were grouped as follows: Ht (Haplotype) 1: C107; Ht 2: C 224; Ht 3: C 225, C 366; Ht 4: C 258; Ht 5: C 259, L125, L174, L175, L216; Ht 6: C 261; Ht 7: C 265; Ht 8: C 287, C 322, C 323, C 25; Ht 9: C 330, C 34, L18, L84; Ht 10: C 22; Ht 11: C 16; Ht 12: C 26, L 92; Ht 13: L7, L 170; Ht 14: L 21; Ht 15: L 24; Ht 16: L 32; Ht 17: L 105; Ht 18: L 107; Ht 19: L 116; Ht 20: L 121; Ht 21: L 172; Ht 22: L 224. The Median Network was calculated with Network 2.0^{15,16} which is available at: <http://www.fluxus-engineering.com>.

Table 2 Variant sites found in mtDNA coding region from centenarians (L)

| Cambr | 12S | | 2158 T | 2392 T | 16S | | 2789 C | 3010 G | ND1 4216* T | ND2 | | A 5633 C | COI 7028 C** | S 7476 C |
|-------|------------|-------------|---------------------|------------|--------------|-------------|------------|--------------|-------------------|-------------|-----------------|----------------|--------------------|----------------|
| | 750 A** | 1438 A** | | | 2404 T | 2706 A** | | | | 4769 A** | 5460 G | | | |
| L170 | G | G | C | - | - | G | - | A | C | G | A | - | T | - |
| L21 | G | G | C | - | - | G | - | A | C | G | A | - | T | - |
| L92 | G | G | - | C | - | G | - | - | C | G | - | - | T | T |
| L107 | G | G | - | - | - | G | - | A | C | G | - | - | T | - |
| L116 | G | G | - | - | - | G | - | - | C | G | - | T | T | T |
| L24 | G | G | - | - | - | G | - | - | C | G | - | T | T | T |
| L32 | G | G | - | - | - | G | - | A | C | G | - | - | T | - |
| L84 | G | G | - | - | C | G | - | - | C | G | - | T | T | T |
| L18 | G | G | - | - | - | G | T | - | C | G | - | T | T | T |
| Cambr | COII | | NC 8269 | ATPase 6 | | 8860 A** | 9656 T | COII 9872 | ND3 | | 10398* A | 10410 T | R 10454 T | |
| | 7786 C | 7789 G | | 8557 G | 8738 T | | | | 8760 T | 10172 G | | | | 10389 T |
| L170 | - | - | A | A | - | G | - | - | - | - | G | C | - | |
| L21 | - | - | A | A | - | G | - | - | - | - | G | - | - | |
| L92 | - | A | - | - | - | G | - | - | - | - | G | - | - | |
| L107 | T | - | - | - | C | G | - | - | - | - | G | - | - | |
| L116 | - | - | - | - | - | G | - | - | A | - | G | - | C | |
| L24 | - | - | - | - | - | G | C | - | A | - | G | - | - | |
| L32 | - | - | - | - | - | C | G | - | - | - | G | - | - | |
| L84 | - | - | - | - | - | G | - | - | A | C | G | - | - | |
| L18 | - | - | - | - | - | G | - | G | A | - | G | - | - | |
| Cambr | ND4L | | 11377 | ND4 | | 12358 | 12612 | 12903 | ND5 | | 13821 | 13879 | 13934 | |
| | 10499 A | 11251 A | | 11506 T | 11719 G** | | | | 12007 G | 13708* G | | | | 13722 A |
| L170 | - | G | - | - | A | A | - | G | - | A | - | C | - | |
| L21 | - | G | - | - | A | A | - | G | - | A | - | C | - | |
| L92 | G | G | A | - | A | - | - | G | - | A | G | - | - | |
| L107 | - | G | - | - | A | - | - | G | - | A | - | - | T | |
| L116 | - | G | - | - | A | - | - | G | C | A | - | - | - | |
| L24 | - | G | - | - | A | - | - | G | - | A | - | - | - | |
| L32 | - | G | - | C | A | - | G | G | - | A | - | - | T | |
| L84 | - | G | - | - | A | - | - | G | - | A | - | - | - | |
| L18 | - | G | - | - | A | - | - | G | - | A | - | T | - | |
| Cambr | ND5 | | ND6 14766 C** | 14798 T | 15067 T | 15227 G | Cytb | | 15788 A | 15812 G | T 15942 T | | | |
| | 14133 A | 14554 A | | | | | 15257 G | 15326 A** | | | | 15452* C | | |
| L170 | - | - | T | - | C | - | - | G | A | - | - | - | | |
| L21 | - | - | T | - | - | - | - | G | A | G | - | - | | |
| L92 | G | - | T | - | - | - | A | G | A | - | - | - | | |
| L107 | - | - | T | C | - | A | - | G | A | - | - | - | | |
| L116 | - | - | T | - | - | - | A | G | A | - | A | C | | |
| L24 | - | G | T | - | - | - | A | G | A | - | A | - | | |
| L32 | - | - | T | C | - | - | - | G | A | - | - | - | | |
| L84 | - | - | T | - | - | - | A | G | A | - | A | - | | |
| L18 | - | - | T | - | - | - | A | G | A | - | A | - | | |

*J-haplogroup missense mutations. **Distinctive variants and rare polymorphisms of the Cambridge (Cambr) reference sequence.^{18,19} 12S, 12 S rRNA; 16S, 16 S rRNA; ND1, NADH dehydrogenase 1; ND2, NADH dehydrogenase 2; A, tRNA-Alanine; COI, Cytochrome c oxidase I; S, tRNA-Serine; COII, Cytochrome c oxidase II; NC, non coding Z; ATPase 6, ATP synthase 6; COIII, Cytochrome c oxidase III; ND3, NADH dehydrogenase 3; R, tRNA-Arginine; ND4L, NADH dehydrogenase 4L; ND4, NADH dehydrogenase 4; ND5, NADH dehydrogenase 5; ND6, NADH dehydrogenase 6; Cytb, Cytochrome b; T, tRNA-Threonine.

in the three-dimensional structure of the relevant tRNAs, and affect their functionality. The mutations 15257A (Asp₁₇₁>Asn₁₇₁) and 15812A (Val₃₅₆>Met₃₅₆) occur in the cytochrome b gene. The first one changes a highly conserved aminoacid, and probably affects the efficiency of this key electron carrier.²¹

Discussion

In order to better understand how mtDNA haplogroup J could be associated to longevity⁸ we have sequenced the mtDNA-D-loop in a sample of centenarians and young controls plus the coding mtDNA region in a subset of centenarians, all categorized as J.

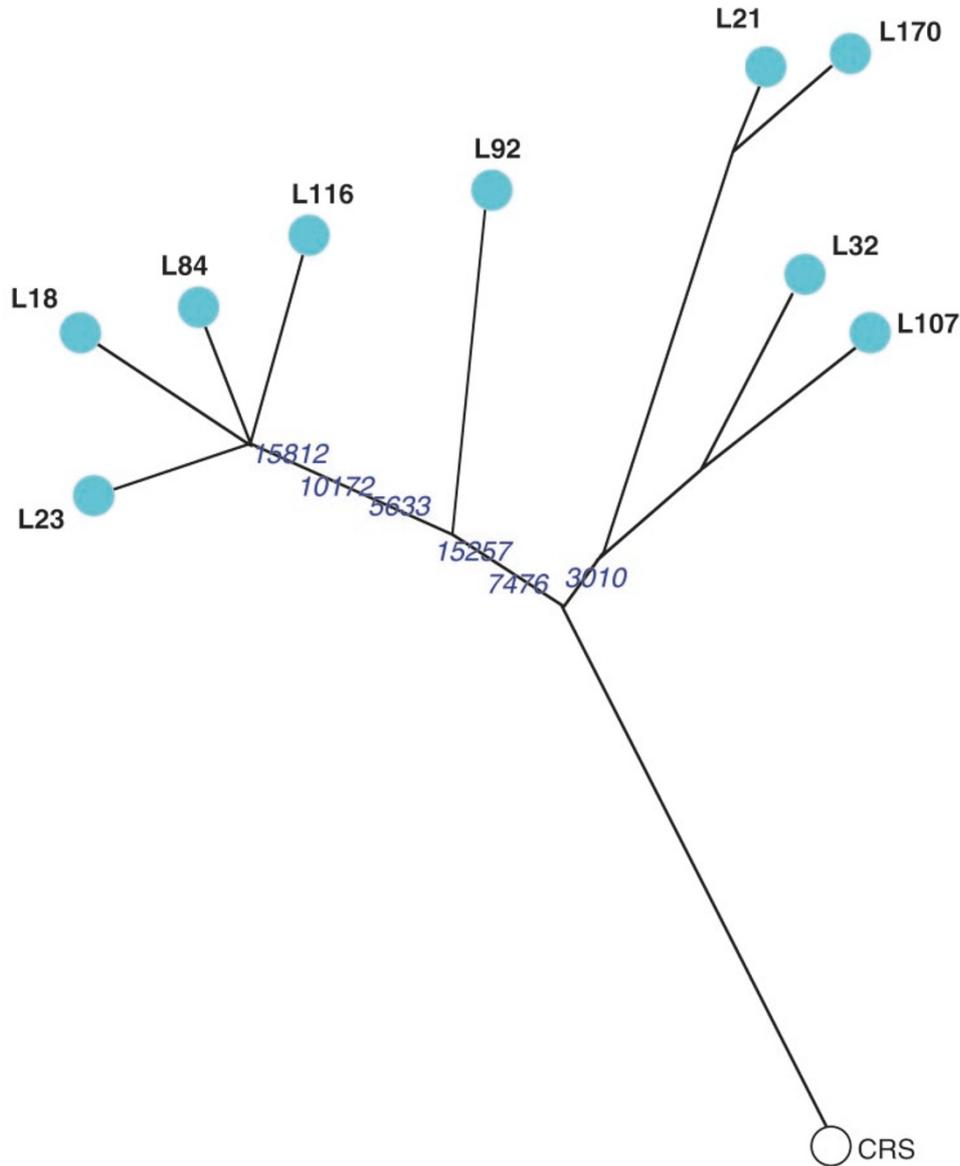


Figure 2 Median Network of the mitochondrial coding region sequences in centenarians (L). The mutations designating the two major branches are shown in italics. The position of the mutations are given according to Anderson *et al.*¹⁸ CRS denotes Cambridge Reference Sequence.

At the analysis of the D-loop (Table 1 and Figure 1) the centenarians did not show any reduction in variation, and actually they were even more diverse than controls, thus ruling out the possibility that, by chance, we sampled a group of closely related centenarians. In addition, the lack of clustering among centenarians indicated that the high frequency of J is not due to a specific haplotype falling in this group but to mutations which characterize the whole, or at least a large part of, haplogroup.

The two branches of the network constructed with the coding region sequence (Table 2 and Figure 2), are intriguingly

characterized by mutations previously associated to complex diseases where mtDNA involvement has been proposed. An exciting example is given by the mutation 3010A that defines one of the two branches of Figure 2. This mutation has been found associated with longevity not only in Italians (present study), but also in Japanese.⁴ However the 3010A occurs also in patients affected by mitochondrial pathologies, even if other mutations have been proposed to be the pathogenic ones.^{23–26} Moreover, the complete sequence of mtDNA in a family with LHON²⁷ showed that it was characterized by the 3010A, besides the causative

mutation at 11778. Similarly, the haplotype defined by the mutations 5633T, 7476T, 15812A has been reported as risk factor in Alzheimer's disease.²⁸ On the whole, the general picture which emerges from these results is that the J haplogroup of centenarians is surprisingly similar to that found associated with several complex diseases.^{11,12,28,29}

How could this apparent paradox be explained? The non-random associations recently observed between mitochondrial and nuclear polymorphisms in some complex traits^{30,31} give some hints, suggesting that complex interactions between mtDNA and nuclear genes play a role on the susceptibility to develop longevity or disease. Indeed a relationship exists between oxidative phosphorylation (OXPHOS) performance and mtDNA haplogroups, with haplogroup T showing a significantly less efficient OXPHOS with respect to H.³² The mutations defining haplogroup J occur in complex I protein subunits and are partly shared with haplogroup T. Previously it has been proposed that such mutations could impair the OXPHOS performance, putting the cell in a vulnerable situation where a further mutation (such as the LHON at 11778) would be more likely to be harmful.^{11,33} However, a low OXPHOS performance may not necessarily be detrimental for the cell. In fact, it was recently shown that an OXPHOS decrease produces increase of Reactive Oxygen Species (ROS), which in turn induce, in a tissue specific way, over-expression of nuclear genes coding for detoxifying enzymes.³⁴ Similar results had been previously reported also in yeast, where it was observed that mutant strains of *Saccharomyces cerevisiae* carrying mtDNA damages were able to up-regulate the expression of stress-responder genes, leading the mutant strains to live longer than the normal ones.³⁵ In this scenario, a certain mtDNA inherited mutation could induce longevity or disease according to the individual-specific genetic background as well as to stochastic events.

The case of the mtDNA J haplogroup is not the first apparent paradox found while studying centenarians, where alleles supposedly risk-bearer for many genes^{36–41} have been found at frequencies similar, or even higher, to those found in the younger population. In any case, the mtDNA sequence analyses carried out in subjects characterized by successful aging and longevity reveal another paradox of centenarians and confirm the complexity of the longevity trait.

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